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Comparison of polymerase chain reaction methods and plating for analysis of enriched cultures of *Listeria monocytogenes* when using the ISO11290-1 method

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ABSTRACT

Analysis for *Listeria monocytogenes* by ISO11290-1 is time-consuming, entailing two enrichment steps and subsequent plating on agar plates, taking five days without isolate confirmation. The aim of this study was to determine if a polymerase chain reaction (PCR) assay could be used for analysis of the first and second enrichment broths, saving four or two days, respectively. In a comprehensive approach involving six European laboratories, PCR and traditional plating of both enrichment broths from the ISO11290-1 method were compared for the detection of *L. monocytogenes* in 872 food, raw material and processing environment samples from 13 different dairy and meat food chains. After the first and second enrichments, total DNA was extracted from the enriched cultures and analysed for the presence of *L. monocytogenes* DNA by PCR. DNA extraction by chaotropic solid-phase extraction (spin column-based silica) combined with real-time PCR (RTi-PCR) was required as it was shown that crude DNA extraction applying sonication lysis and boiling followed by traditional gel-based PCR resulted in fewer positive results than plating. The RTi-PCR results were compared to plating, as defined by the ISO11290-1 method. For first and second enrichments, 90% of the samples gave the same results by RTi-PCR and plating, whatever the RTi-PCR method used. For the samples that gave different results, plating was significantly more accurate for detection of positive samples than RTi-PCR from the first enrichment, but RTi-PCR detected a greater number of positive samples than plating from the second enrichment, regardless of the RTi-PCR method used. RTi-PCR was more accurate for non-food contact surface and food contact surface samples than for food and raw material samples especially from the first enrichment, probably because of sample matrix interference. Even though RTi-PCR analysis of the first enrichment showed less positive results than plating, in outbreak scenarios where a rapid result is required, RTi-PCR could be an efficient way to get a preliminary result to be then confirmed by plating. Using DNA extraction from the second enrichment broth followed by RTi-PCR was reliable and a confirmed result could be obtained in three days, as against seven days by ISO11290-1.

1. Introduction

The detection time of pathogen presence in foods and food processing environments is often crucial as it has a direct consequence on the reaction time for decision-making related to risk management and/or risk communication. In this context, speeding up the methods for pathogen detection became a goal of many research teams. This especially applies to the detection of *Listeria monocytogenes*, a Gram-positive, rod-shaped bacterium well-known to be the causative agent of listeriosis in humans. Owing to its elaborate physiological adaptation mechanisms, *L. monocytogenes* can survive and even proliferate under adverse environmental conditions such as low pH, high salinity, low temperature and the

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presence of detergents (Khelef et al., 2006; Pricope et al., 2013). *L. monocytogenes* occurrence in the food industry and food products is a major public health concern, especially with recent listeriosis outbreaks for example in Canada (Gaulin et al., 2012), USA (MMWR, 2011) and Austria, Germany and Czech Republic (Fretz et al., 2010; Schoder et al., 2012). *L. monocytogenes* is ubiquitous in the environment and has been isolated from a wide variety of ready-to-eat foods, such as meat, fish, vegetables and dairy products (Lianou and Sofos, 2007). Its presence in food processing facilities has been described in meat-producing plants (Chasseignaux et al., 2002; Peccio et al., 2003), in dairy processing environments (Alessandria et al., 2010; Fox et al., 2011) and in fish-producing facilities (Chen et al., 2010; Di Ciccio et al., 2012).

While testing food products for the presence of pathogenic microorganisms is basic to ensure food safety, for pathogens such as *L. monocytogenes* that can persist in food processing facilities for a long time and result in recurring product contamination, testing of the processing environment is critical. Sampling and testing of the processing environment is an effective way to assess hygiene and prevent future contamination events (Tompkin, 2002). A stringent *L. monocytogenes* control programme is essential even in small processing facilities, along with measures to prevent and control persistent contamination in niches in processing facilities. Sampling and testing for *L. monocytogenes*, on a routine basis according to a sampling scheme of the processing areas and equipment is mandatory, as stipulated by the EC Regulation 2073/2005 defining microbiological criteria for foods and amended by Regulation 1441/2007 (Anonymous, 2005, 2007).

The ISO11290-1 is the standard European method for the detection of *L. monocytogenes* in food and environment samples (Anonymous, 1996). It is based on a two-step enrichment, the first 24-hour step being performed with half-Fraser broth for recovery of cells and the second 48-hour step being performed with full-Fraser broth for selective multiplication of *L. monocytogenes* cells. Both cultures in half-Fraser and full-Fraser broths are plated onto agar in order to detect positive *L. monocytogenes* colonies, which have to be further confirmed. Thus, this method is time-consuming and results cannot be obtained in less than five days. In the case of a *L. monocytogenes* outbreak, the delay in obtaining the results slows down the process of detecting the presence of *L. monocytogenes* and the implementation of corrective actions to maintain the safety of the food products and to protect consumer health. The implementation of molecular methods to the field of food pathogen detection supports traditional microbiological methods and increases the consistency of the results (Rossmanith and Wagner, 2011). For example, some studies have been carried out on the detection by real-time PCR (RTi-PCR), sometimes along with quantification, of *L. monocytogenes* in food samples (Aparecida de Oliveira et al., 2010; O'Grady et al., 2008; Oravcová et al., 2007; Rodríguez-Lázaro et al., 2004; Schoder et al., 2012; Stevens and Jaykus, 2004). Postollec et al. (2011) reported several studies based on food sample enrichment and quantitative PCR for the detection of *L. monocytogenes* with quantitative PCR detection thresholds close to those obtained with standard plate counts. These studies focused mainly on food analysis and more specifically on artificially contaminated foods. A lack of data seems to exist regarding the effort to reduce the time of analysis for naturally contaminated food and processing environment samples. The aim of the present study was to improve the time-to-result for the rapid, relatively simple and accurate detection of *L. monocytogenes* in naturally contaminated food, processing environment and raw material samples by combining the ISO11290-1 method and PCR.

2. Materials and methods

2.1. Sampling plan

Samples including non-food contact surfaces (NFCSs), food contact surfaces (FCSs), foods and raw materials were collected from 13 different dairy and meat food processing facilities located in Austria, Greece, Ireland, Romania, Slovakia and Spain. NFCS samples were taken on areas such as floors, walls, drains and equipment wheels, while FCS samples were taken on conveyors, belts, tables, slicers, etc. Raw materials included products such as raw cow and ewe milk, raw sheep and pork meat, brine, ewe and cow milk cheese curd, ripening liquid for pork jaw, and chicken skin. Foods included meat products (salami, sausages, tenderloin, smoked bacon and pork jaw, pork hot dog, chicken burger and drumstick) and different types of cheese (Cheddar cheese, farmhouse cow milk semi-soft cheese, pasteurized ewe milk cheese, non-ripened soft cheese from unpasteurized ewe milk [Bryndza cheese], smoked cheese). For each laboratory, the number of samples and the facility types are described in Table 1. Swab samples were collected using pre-moistened sterile sponge-sticks (3M, St. Paul, Minnesota, USA). Liquid samples were collected using sterile dippers. All samples were collected wearing gloves and appropriate protective clothing, individually packaged to prevent cross-contamination, placed in a cool box with ice packs and transported directly to each partner laboratory where they were analysed within 2 h including transport.

2.2. Listeria detection by ISO11290-1 and identification of isolates

The two-step enrichment method ISO11290-1 was used for the detection of *L. monocytogenes* in all the samples (Anonymous, 1996), with two minor exceptions. Firstly, in four countries, only ALOA (Oxoid, Hampshire, UK or Merck KgA, Darmstadt, Germany) agar was used while in the other two countries PALCAM (Biokar, Pantin, France) agar and ALOA agar were used. Secondly, 20 µl of enriched broths were spread on agar plates after each enrichment step, and incubated for 48 h at 37 °C. As ALOA agar is more confirmatory for *L. monocytogenes* strains (Gracieux et al., 2003), especially for low-virulence strains found in the processing environment (Roche et al., 2009a,b), up to five presumptive-positive *L. monocytogenes* colonies (blue–green with a surrounding halo) were isolated when possible from ALOA plates of the first and second enrichments. All isolates were confirmed as *L. monocytogenes* using PCR as described previously by Border et al. (1990) and Bubert et al. (1999) for Austria, by Rodríguez-Lázaro et al. (2004) for Greece, Ireland and Spain and by Oravcová et al. (2006) for Romania and Slovakia. The sample was declared positive for *L. monocytogenes* if any of the isolates were confirmed.

Table 1
Number of samples and facility types for each country.

		AT	GR	IE	RO	SK	ES
Processing facility type		Cheese	Cheese	Cheese	Meat	Cheese	Cheese and poultry meat
Sample type	NFCS[a]	32	80	80	40	137	45
	FCS[b]	0	87	20	41	85	31
	Food	0	30	14	20	46	23
	Raw materials	0	11	6	10	32	2
Number of samples		32	208	120	111	300	101

[a] Non-food contact surface.
[b] Food-contact surface.